



# Is endoscopic inspection necessary to detect residual disease in acoustic neuroma surgery?

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## Abstract

**Main goals** To analyze how and when the endoscope is used in vestibular schwannoma surgery and identify the benefits of using endoscopy in this type of surgery.

**Background** It is currently unclear if there is any benefit from using an endoscope in vestibular schwannoma surgery so this retrospective analysis set out to study this.

**Methods** All the patients who underwent vestibular schwannoma surgery at our clinic were included for all the vestibular schwannoma approaches taken. We studied when endoscopy was used during surgery and the goal of using endoscopy. Several pre- and postoperative factors were assessed such as complications, facial function, and hearing function in the case of techniques that allow hearing preservation.

**Results** From January 2015 to September 2018, 280 patients underwent lateral skull base surgery. Of these, 112 were included in this study. The endoscope was used in all 112 patients, and in eight cases it was possible to identify residual disease using the endoscope to check the surgical field, and then to remove the disease under endoscopic view. Moreover, in two other cases, the endoscope was used to resolve a vasculoneural conflict between the anterior inferior cerebellar artery (AICA) loop and facial nerve in one case, and for deafferentation of the superior and inferior vestibular nerves in the second case. No major intraoperative complications occurred in our series. There was no statistically significant difference in post-operative facial nerve function between patients in whom the endoscope was used as a diagnostic tool and patients in whom it was used as an operative tool ( $p=0.3152$ ).

**Conclusions** The endoscope may be useful, especially in surgical techniques where there is poor control of the internal auditory canal (IAC). An endoscopic support technique is strongly recommended to avoid residual disease, particularly in retrosigmoid and retrolabyrinthine approaches. Moreover, the recent introduction of the transcanal transpromontorial approach allows the endoscope to be used during all the procedures in patients affected by a vestibular schwannoma limited to the IAC or to support surgical procedures during an enlarged microscopic approach.

**Keywords** Vestibular schwannoma residual · Acoustic neuroma Endoscopic surgery · Vestibular neuroma surgery · Lateral skull base approaches

## Introduction

Vestibular schwannomas are benign, slow growing tumors and they represent the most common cerebellopontine angle (CPA) lesions. Different therapeutic options are possible in the management of this pathology, based on the patient and tumor characteristics: wait-and-scan, radiosurgery and microsurgery. Focusing on the surgical treatments, the goal of the operation is achieving total removal of the lesion, preserving the integrity of the facial nerve, avoiding injuries to the vasculoneural structures in the CPA, and when possible, preserving a serviceable hearing

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[1, 2]. The classic microsurgical approaches developed during recent decades include the retrosigmoid approach, the translabyrinthine approach, and the middle cranial fossa approaches. The main drawback of the posterior fossa approach using the retrosigmoid corridor is the inadequate control over the fundus of the internal auditory canal (IAC) increasing the chances of leaving residual disease in this anatomical site [2–5].

The use of the endoscope in the retrosigmoid approach was introduced in recent decades as an adjunctive tool in microsurgery to overcome this problem. The advantages of the endoscope are its improved visualization of anatomical structures of the CPA, minimizing cerebellar retraction, and a reduction in the rate of complications [2, 5]. More recently, a minimally invasive approach has been proposed with a fully endoscopic resection of the tumor via a retrosigmoid keyhole craniotomy [6–8]. Furthermore, in recent years, a transcanal transpromontorial approach was developed to reach the inner ear and the CPA through the external auditory canal. The advantages of this approach are the direct visualization of the IAC with minimal temporal bone drilling, without craniotomy and manipulation of the dura and, therefore, lower morbidity compared with classic microsurgical approaches.

Despite the advent of endoscopic approaches in the otological field, the microscope is still the dominant tool in vestibular schwannoma surgery. Although the procedures for vestibular schwannoma surgery are well codified, there is a possibility of leaving residual diseases in the IAC fundus, porus, or CPA. Through a retrospective analysis, our study evaluated the prevalence of residual disease after vestibular schwannoma removal using a microscopic surgical approach, and with an endoscopic check of the surgical cavity at the end of the removal procedure. The aim is to clarify whether the use of the endoscope is necessary for optimization of surgical radicality.

## Materials and methods

### Inclusion criteria

From January 2015 to September 2018, 112 patients underwent microscopic surgery for vestibular schwannoma (translabyrinthine, transcanalar transpromontorial, retrolabyrinthine, retrosigmoid, transotic and middle cranial fossa approaches) at the Otolaryngology Department of the University Hospital of Verona.

A retrospective chart review was undertaken to identify those patients in whom the endoscope was used during surgery.

### Exclusion criteria

The following patients were excluded from this retrospective analysis.

1. Patients who underwent vestibular schwannoma surgery and who were not evaluated endoscopically at the end of the surgical procedure for a final check of the surgical cavity.
2. Patients who underwent transcanal exclusive endoscopic surgery for small vestibular schwannoma removal in the IAC.
3. Patients undergoing lateral skull base surgery for lesions other than the vestibular schwannoma and also patients affected by type 2 neurofibromatosis.
4. Patients affected by vestibular schwannoma who underwent a wait-and-scan policy or Gamma knife therapy.

### Indications for surgery

The choice of surgery varied depending on the tumor extent, its location, and the preoperative audiological status of the patient, as follows:

- In patients affected by vestibular schwannoma with IAC involvement and with or without extension to the CPA (Koos grade I–III) [10] in association with a poor hearing function (Class C–D according to the AAO-HNS classification [11]), a transcanal transpromontorial approach was used.
- In patients affected by vestibular schwannoma located in the IAC with CPA involvement (Koos grade III–IV), a translabyrinthine approach was adopted, regardless of hearing function.
- Patients affected by vestibular schwannoma limited to the CPA (Koos grade II–IV) with or without minimal extension to the IAC, with the possibility of hearing preservation (Class A–B according to AAO-HNS classification), underwent a retrosigmoid approach.
- Patients affected by small-medium sized vestibular schwannoma (1.5–2 cm) limited to the CPA without extension to the IAC, with a good preoperative hearing function (Class A–B according to AAO-HNS) and anatomically favorable hyperpneumatized temporal bone, underwent a retrolabyrinthine approach.
- In the case of giant vestibular schwannomas with anterior extension to the CPA or with petrous apex involvement, a transotic approach was performed.

## Operative setup and instrumentation during endoscopy

In total, 112 patients were evaluated retrospectively. They all underwent vestibular schwannoma removal using a microscopic approach, and this was followed by an endoscopic check of the final surgical cavity to rule out the presence of any residual disease. For all of the procedures, rigid endoscopes were used with a diameter of 4 mm and angled at 0° or 45° (Karl Storz Tuttlingen, Germany). The 70° endoscope was only used in the case of evidence of a residual tumor which was impossible to manage using a 45° endoscope.

After the microscopic surgical excision of the vestibular schwannoma, a rigid endoscope (0° or 45°) was introduced into the surgical cavity to check the CPA and IAC for any residual disease. Endoscopic magnification of the entry zone, the acousticofacial bundle, and the entire IAC from the fundus to the porus was systematically performed.

In the case of a remnant tumor, an angled endoscope was used operatively to remove the disease. The endoscope was held by the operating surgeon and a high definition monitor was placed on the contralateral side of the patient, in direct line-of-sight of the primary surgeon. The surgeon's dominant hand held the operative instrument/dissector while he watched the monitor.

## Data examined

We evaluated the percentage of residue disease detected by endoscopy after the microscopic approach and the feasibility of residual tumor removal under endoscopic view. We also evaluated pre- and postoperative facial nerve function according to the House–Brackmann scale (HB) [9]. We analyzed the facial outcome for the group of patients in whom the endoscope was used operatively and the group in whom the endoscope was only used for final inspection of the surgical cavity.

## Results

From January 2015 to September 2018, 205 patients underwent lateral skull base surgery. From these patients, 112 were included in this study, according to the inclusion criteria. Of these, 41 patients were male and 71 female, and in 59 patients the side involved by the tumor was the right side and in 53 patients, it was the left side.

Of the 112 patients, 18 underwent a retrosigmoid approach, 41 a translabyrinthine approach, 47 patients underwent an enlarged transcanal transpromontorial approach, 4 a transotic approach, 1 a retrolabyrinthine approach, and 1 a

**Table 1** Patient demographic data and tumor classification

Number of patients	112
<i>Gender</i>	
Male	41 (36.6%)
Female	71 (63.4%)
<i>Side</i>	
Right	59 (52.7%)
Left	53 (47.3%)
<i>Tumor classification (Koos)</i>	
Koos I	26 (23.2%)
Koos II	34 (30.4%)
Koos III	28 (25.0%)
Koos IV	24 (21.4%)

**Table 2** Surgical approaches and residual disease found at the endoscopic check of the surgical field

Surgical approach	Residual disease, <i>n</i> (%)	
Retrosigmoid approach	18 (16.1%)	4 (22.2%)
Translabyrinthine approach	41 (36.6%)	0
Enlarged transcanal approach	47 (42.0%)	3 (6.4%)
Transotic approach	4 (3.6%)	0
Retrolabyrinthine approach	1 (0.9%)	1 (100%)
Middle cranial fossa approach	1 (0.9%)	0

middle cranial fossa approach (Table 1). In 8 patients out of 112, residual disease was found during the endoscopic check of the surgical field after the microscopic step.

## Retrosigmoid approach

In 4 out of 18 cases (22.2%) using the retrosigmoid approach, the endoscope permitted identification of residual disease once the microscopic step had been completed (Table 2). In all four cases, the residual disease was found in the IAC. In these four patients, the tumor reached the fundus of the IAC; therefore, the endoscope was used as an operative tool to remove the residual disease. In all these cases, a 45° endoscope was used to achieve complete visualization of the fundus of the IAC as this was not possible with a 0° endoscope. It was also used for removal of the residual tumor.

In 14 out of 18 cases (77.8%) using retrosigmoid surgery, no residual tumor was found during the endoscopic check. In these cases, the tumor was located only in the CPA or had limited extension to the medial portion of the IAC. In one case (5.5%), during endoscopic exploration of the surgical cavity, cellularity of the petrous bone was found which gave rise to a connection between the CPA and the petrous bone air system. This connection was removed under endoscopic

view by obliterating those cells with a fragment of temporal muscle and fibrin glue.

An endoscopic view of the anatomical structures of the CPA and of the fundus of the IAC is shown in Fig. 1.

### Enlarged transcanal transpromontorial approach

In 47 out of 112 cases, an enlarged transcanal transpromontorial approach was performed. In all these cases, 0° and 45° endoscopes allowed us to check the final surgical cavity and obtain a wider view of the nervous and vascular structures in the CPA to detect any residual tumor as demonstrated in Fig. 2.

In 3 out of the 47 patients (6.4%) in whom the enlarged transcanal approach was employed, we were able to find residual disease during the endoscopic check. In these three cases, the tumor was not located in a straight line with the EAC, so the straight microscopic view was not sufficient to remove the anterior portion of the tumor. An example of one of these cases is shown in Fig. 3 in which it is possible to see a vestibular schwannoma with anterior extension to the petrous apex. In these cases, a 45° endoscope was used as an operative tool to achieve a complete view of the surgical field and to remove the residual tumor. It was not possible to use a 0° endoscope because an angled instrument was required to view and remove the tumor. Using the angled endoscope (45°), it was possible to dissect the

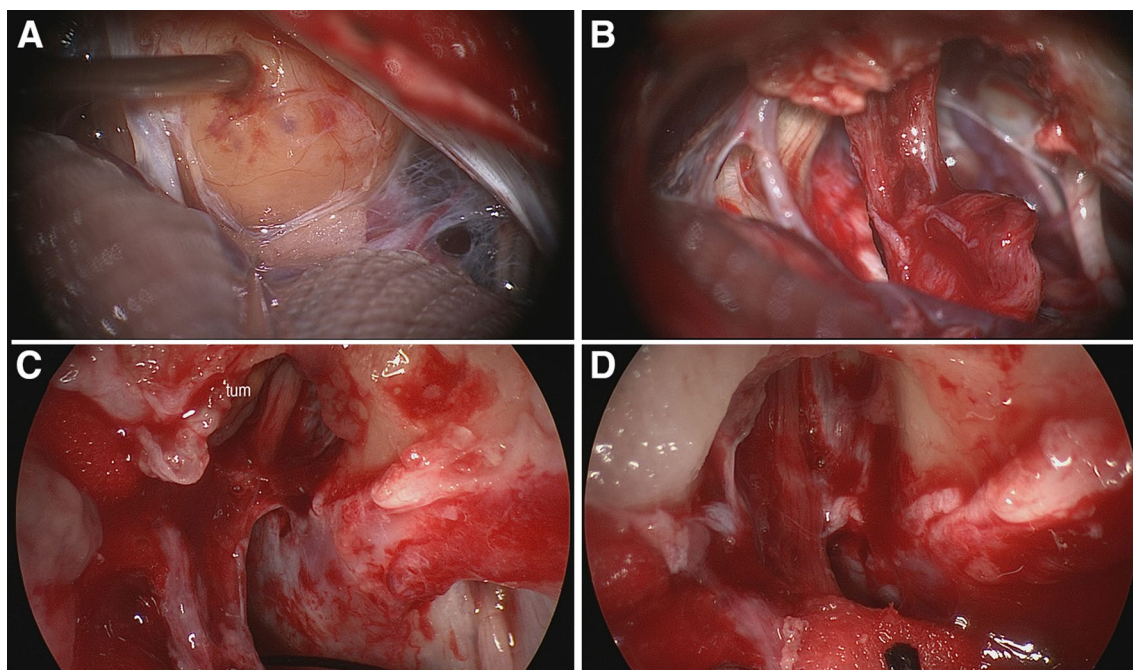
residual tumor in the petrous apex, medial to the internal carotid artery (ICA). In one case, at the end of microscopic tumor removal, endoscopy allowed us to detect a residual tumor with a medial extension with respect to the intralabyrinthine tract of the facial nerve.

### Translabyrinthine approach

In 41 out of 112 cases, a translabyrinthine approach was adopted. At the end of the microscopic step, the endoscopic check of the surgical field did not identify any residual disease. Nevertheless, in one case, the endoscope was used to perform a surgical correction of a neurovascular conflict between the anterior inferior cerebellar artery and the facial nerve, which was identified at the end of tumor removal. In all the other translabyrinthine approaches, a fully microscopic approach was performed, since this was sufficient to reach and completely remove the lesion from the fundus of the IAC to the CPA.

### Retrolabyrinthine approach

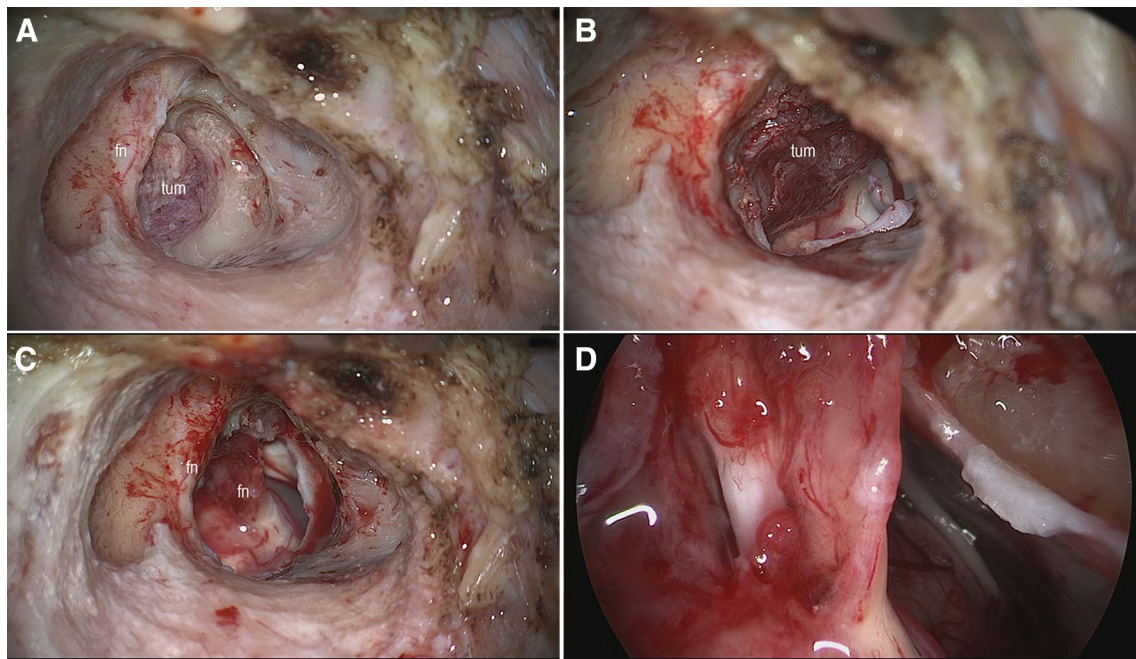
A retrolabyrinthine endoscopic-assisted approach was adopted in only 1 case, because of the location and size of the schwannoma (20 mm size, with extension to the CPA and minimal involvement of the medial portion of the IAC). The 70° angled endoscope was used to evaluate the final



**Fig. 1** Right side, retrosigmoid approach. **a** Microscopic view: the acoustic neuroma in the cerebellopontine angle is identified. **b** Microscopic view: the cerebellopontine portion of the tumor is removed. Residual disease is identified in the internal auditory canal (IAC). **c**

Endoscopic view: identification of the acoustic–facial bundle entering the IAC and the intracanalicular residual tumor. **d** The residual tumor in the IAC is removed endoscopically, preserving the facial and cochlear nerve. *tum* tumor

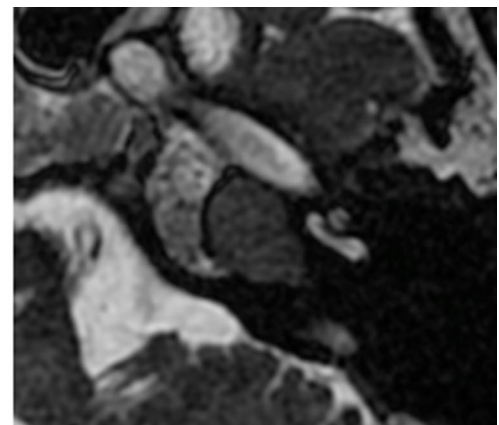
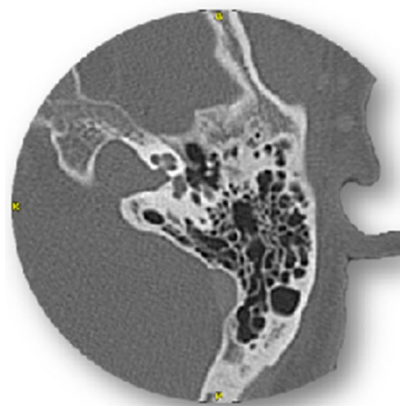




**Fig. 2** Right side, transcanalar approach. **a** The IAC is exposed microscopically through a transcanal transpromontorial approach. **b** Microscopic view: the cerebellopontine angle is exposed and the tumor is detected from the fundus to the entry zone in the brain stem.

**c** Tumor removal under microscopic view with preservation of the facial nerve. **d** Endoscopic check of the final surgical cavity; no identification of residual disease, with preservation of the facial nerve. *tum* tumor, *fn* facial nerve

**Fig. 3** Left panel: coronal temporal bone CT scan. Right panel: coronal MRI of the same patient. The radiological images show a vestibular schwannoma with an anterior extension to the petrous apex beneath the internal carotid artery



surgical cavity at the end of the microscopic dissection and allowed the identification of a residual tumor at the IAC porus, which was removed using angled instruments under endoscopic view as shown in Fig. 4.

### Transotic approach

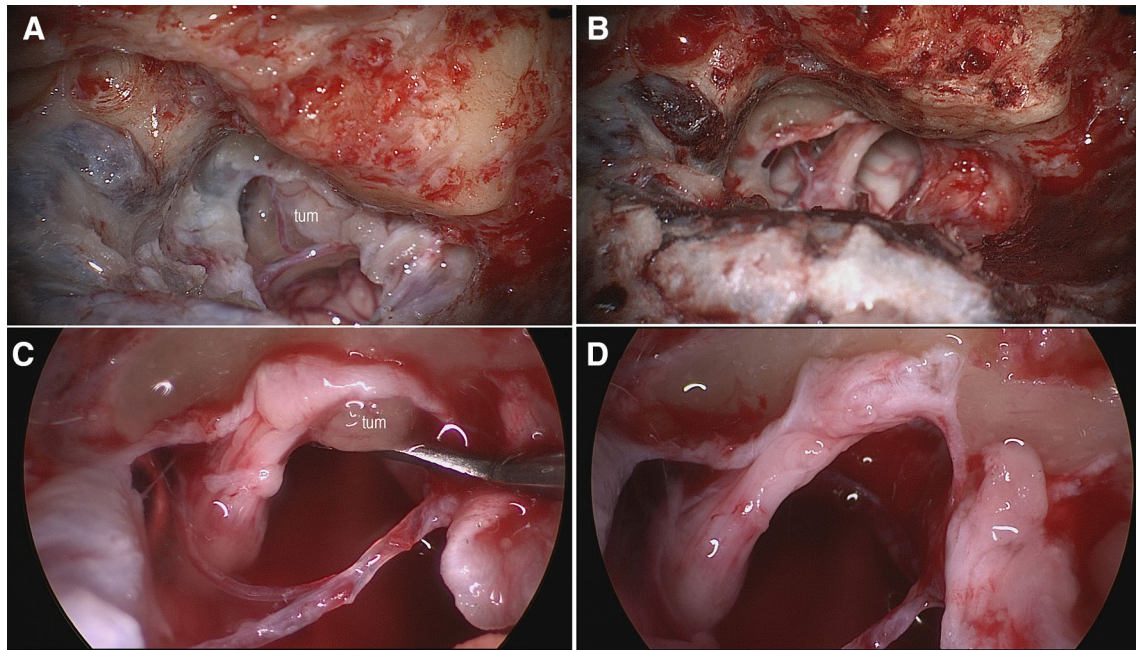
In 4 patients, a transotic approach was performed for bulky vestibular schwannomas, Koos grade 3 or 4, involving the IAC and the petrous apex, extending also to the CPA anteriorly, with troncoencephalic compression. In these cases, no residual tumor was found at the endoscopic check.

### Middle cranial fossa approach

In one case, a middle cranial fossa approach was performed. The tumor was completely removed under microscopic view. In this case, the endoscopic check did not demonstrate any residual disease.

### Outcomes

Anatomic preservation of the facial nerve was achieved in 112/112 cases (100%). Preoperatively, 105 out of 112 (93.7%) patients presented a normal facial nerve function,



**Fig. 4** Left side, retrolabyrinthine approach. **a** Microscopic view: the acoustic neuroma is exposed through a retrolabyrinthine approach, preserving the labyrinth. **b**. Microscopic view: the tumor is removed microscopically. **c** Endoscopic check of the final surgical cavity with

identification of residual tumor in the fundus of the IAC. **d** Endoscopic removal of the residual tumor in the fundus of the IAC, behind the posterior labyrinth, preserving the facial and cochlear nerves. *tum* tumor

while 4 (3.6%) patients showed a grade II, 2 (1.8%) a grade III and 1 (0.9%) a grade IV facial nerve function according to the House–Brackmann scale.

Postoperatively, 86 out of 112 (76.8%) patients maintained a normal facial nerve function, while 12 (10.7%) patients presented a grade II, 7 (6.2%) a grade III, 5 (4.5%) a grade IV, and 2 (1.8%) a grade V facial nerve function according to the House–Brackmann scale. None of the patients had complete facial palsy after the operation.

There was no statistically significant difference in postoperative facial nerve function between the group of patients in whom the endoscope was used as an operative tool and those patients in whom it was only used for radicality assessment after microscopic removal of the tumor (Chi squared test:  $p = 0.3152$ ).

There were no major neurological complications such as quadriplegia, hemiparesis, bacterial or aseptic meningitis, permanent lower cranial nerve deficits, or death. No intra-operative complications were noted.

Regarding postoperative complications, we observed 7 (6.2%) cases of cerebrospinal fluid wound leakage or rhinorrhea. In 5 of these 7 patients, the leakage resolved spontaneously or was treated with lumbar drain placement. In the other two patients, cerebrospinal fluid leakage required surgical repair.

## Discussion

Careful preoperative surgical planning is mandatory in the treatment of vestibular schwannoma, in particular, the choice of surgical route should be tailored, based on the size and location of the tumor, hearing function and characteristics of the patient (age and performance status). For patients affected by vestibular schwannoma, an advanced referral hospital should be able to offer all possible surgical routes to guarantee total removal of the tumor, preserving facial nerve function and, wherever possible, preserving hearing function, with low pre- and postoperative morbidity. Different microscopic surgical routes are adopted in vestibular schwannoma surgery, but not all are able to expose the whole anatomical area where the tumor is located, such as the fundus of the IAC (retrosigmoid, retrolabyrinthine approaches) or the whole CPA (transcanal transpromontorial approach and middle cranial fossa). In recent years, the introduction of endoscopy into common practice during ear surgery has allowed the endoscope to be applied during lateral skull base approaches, as support to the microscope approaches or, in selected cases, as a total endoscopy-based procedure [12].

From our retrospective review, we noted how it was possible to find residual disease in 8/112 cases that would



not otherwise have been identified using the microscopic approach. The number of residual tumors was very variable from technique to technique, but without doubt, the translabyrinthine approach proved to be the best procedure, avoiding leaving residual tumor from the IAC to the CPA and allowing microscopic visualization of the entire surgical field.

The transcanal transpromontorial approach represents a new procedure in the oto-neurosurgical field and it proved effective for removal of vestibular schwannomas located in the IAC; however, in cases of tumor extension under the ICA into the petrous apex, the use of the endoscope may be necessary to remove residual disease.

The use of the endoscope in vestibular schwannoma surgery has been widely discussed in the literature either as support during a microscopic approach or as an exclusive tool in surgery. However, from the literature reports, the endoscope plays a fundamental role in vestibular schwannoma surgery in only some surgical routes, providing valuable assistance to check for any residual diseases in the final surgical cavity and to examine hidden areas that would remain unexplored using the microscope. Regarding the surgical approaches, the translabyrinthine approach allows good exposure of both the CPA and IAC, and the use of the endoscope during this approach is not necessary in the majority of cases. In fact, there are very few articles in the literature which stress the use of endoscopy in this approach. In particular, during a translabyrinthine approach, Wackym and colleagues used 0° and 30° endoscopes to visualize the undersurface of the stapes footplate to confirm that the stapes was not displaced from the oval window and to determine whether there were open air cells that had not been identified with the microscope and sealed with wax [5].

The middle cranial fossa approach is also commonly used in the case of small vestibular schwannomas limited to the IAC, in patients with good preoperative hearing function. This approach allows good control of the entire IAC from the fundus to the porus. A few reports are present in the literature about the use of endoscopy in the middle fossa approach, in particular, the experience of Chen and colleagues [13]. They affirm that dissection toward the fundus is limited by the neighboring cochlea and anterior semicircular canal. For this reason, residual tumors can hide in the area vestibularis (area of the fundus where the superior and inferior vestibular nerves enter the vestibule), out of the direct line of sight of microscopy. With endoscopy, the most lateral extent of the fundus including the transverse crest can be visualized. Resection of tumors from the fundus under the operative microscope sometimes requires their blind dissection. This is even more difficult when the tumor extends inferiorly to the transverse crest, which can sometimes measure up to 3 mm in length [13].

In our series, only one middle cranial fossa approach was adopted, despite this approach being routinely used in our department, especially for petrous apex lesions. In the authors' opinion, endoscopic support should always be considered during the middle cranial fossa approach, especially if the vestibular schwannoma has spread into the petrous apex, running along the horizontal portion of the internal carotid artery. Conversely, in the retrosigmoid approach, the role of endoscopy for microscopic assistance or dissection is acknowledged in the literature.

Many scientific articles in the literature have confirmed the importance of endoscopy during the retrosigmoid approach, total endoscopic procedures or combined microscopic endoscopic-assisted surgery [5–7, 20, 21]. Therefore, in the retrosigmoid approach, exposure of the IAC fundus is incomplete using only the microscope as it requires major drilling of the bone. As a result of the microscopic view and the anatomical conformation of the IAC, residual disease in the fundus requires a blind dissection. For this reason, many authors have stressed the importance of endoscopic-assisted surgery. The use of 45° and 70° angled endoscopes allows management of residual disease in the fundus and removal can easily be performed with endoscopic assistance, carrying out a complete tumor resection and avoiding residual tumor in the fundus [5, 14]. In the opinion of other authors, the use of the endoscope is essential in a key hole technique in the retrosigmoid approach, emphasizing its exclusive use [7, 15].

Few studies are present in the literature on the use of endoscopy in the retrolabyrinthine approach. The main drawback of the presigmoid retrolabyrinthine technique in vestibular schwannoma surgery concerns the poor exposure of the IAC due to the presence of the labyrinthine complex. In the experience of Iacoangeli and colleagues, the endoscope has proved to be essential because, with it, it is possible to respect the neurovascular structures beyond the preserved labyrinthine complex, with visualization of the IAC, removal of the intracanalicular tumor, assessment of the radical schwannoma excision, and prevention of CSF fistula because of the improved view of perimeatal dehiscence air cells [16]. In our department, the retrolabyrinthine approach is used mostly for the removal of petrous apex meningioma with adhesions to the temporal dura.

In our study, we chose a retrolabyrinthine approach in only one case, because of the characteristics, dimensions and location of the vestibular schwannoma. In this case, endoscopic assistance was necessary for removal of the tumor which had limited extension to the CPA and minimal involvement of the medial portion of the IAC. In this case, the use of endoscopy at the end of the microscopic procedure allowed the tumor residual behind the facial nerve in the porus to be detected and better visualized, and allowed its complete removal using a 45° angled endoscope.

The transcanal transpromontorial approach has recently been reported in the literature for the removal of intracanalicular vestibular schwannomas. This approach was codified by our working group and it arose from our endoscopic experience in middle ear surgery [12, 17]. This approach was introduced as a fully endoscopic technique, allowing perfect control of the whole IAC from the fundus to the porus from the external auditory canal, but with poor surgical control of the CPA. For these reasons, this approach was only indicated in patients affected by vestibular schwannoma limited to the IAC requiring surgery, and with poor hearing function.

This technique was then refined and extended (enlarged or expanded transcanal transpromontorial approach) using the microscope, allowing the removal of schwannomas with CPA extension in a straight line with respect to the course of the IAC (Koos II–III) [18].

So in the transcanal transpromontorial approaches, the use of endoscopy can be exclusive or combined with the microscope, ensuring an excellent view from the fundus of the IAC to the porus. These approaches allow excellent exposure and surgical control of the fundus because exposure of the fundus and the whole IAC is an expected step. The IAC is opened through the fundus and then it is completely exposed. Particularly in the transpromontorial approaches, endoscopy was useful mostly in patients affected by a vestibular schwannoma with an anterior development, toward the petrous apex and medial to the intrapetrous tract of the internal carotid artery.

In this study, we retrospectively analyzed only the transcanal transpromontorial microscopic approach and evaluated its efficacy in terms of complete tumor removal. In fact, our study highlights this procedure as an efficient approach for tumor removal from the fundus to the porus, under microscopic vision. We only found poor microscopic visualization in rare cases where the tumor extended to the petrous apex, under the horizontal tract of intrapetrous ICA, making endoscopic assistance necessary.

Despite the recent technological improvements in endoscopy, most lateral skull base surgery continues to be performed using microscopy, and some criticism from the otology community should be considered. Most surgeons have a microscopy-based background and they find it difficult to get used to working with one hand. Essentially, the major concern is the fact that the endoscopic technique requires single-handed use and facial nerve damage could occur during dissection of the tumor from the facial nerve, in particular, stretching of the nerve with possible consequential paralysis.

In fact, in the literature, there are experiences of mechanical damage to the anatomical structures of the CPA because of the absence of three-dimensional vision, with risks of iatrogenic neural or vascular injury [19]. For this reason, the use of endoscopy in the CPA should be performed after appropriate endoscopic training. Among other concerns,

there is also a potential for heat injury from the endoscopic light source, which may cause damage to the nervous tissue of the facial nerve with potential consequential facial paralysis. Despite the fact that thermographic evaluation by Hori et al. [19] did not reveal a significant increase in local temperature using an endoscope, it is possible to avoid any temperature-related problems using frequent irrigation of the surgical field.

Our case series showed an overall good result in terms of facial nerve outcome in the group of patients who underwent a surgical procedure in which endoscopy was only used to explore the surgical cavity and in the group in whom endoscopy was used in an operative way. This is an even more important result given the fact that the endoscope was widely used in the most complex settings where residual disease was found or suspected in a hidden area, due to extension of the disease.

In conclusion, the use of the endoscope may be crucial, especially in surgical techniques where poor control of the IAC is present. An endoscopic support technique is strongly recommended to avoid residual disease, especially in retrosigmoid and retrolabyrinthine approaches.

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## Compliance with ethical standards

**Conflict of interest** The present authors have no financial relationship to disclose.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study. All the authors have read and approved the manuscript.

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